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Epidemiological Reports

An Outbreak of Foodborne Infection Caused by *Shigella sonnei* in West Bengal, India

Falguni Debnath[1], Asish K. Mukhopadhyay[2], Goutam Chowdhury[2], Rudra Narayan Saha[2], and Shanta Dutta[2]*

[1]*Division of Epidemiology, National Institute of Cholera & Enteric Diseases, CIT Rd, Scheme XM; and*
[2]*Division of Bacteriology, National Institute of Cholera & Enteric Diseases, CIT Rd, Scheme XM, Beliaghata, Kolkata -10, India*

SUMMARY: A foodborne acute gastroenteritis outbreak due to *Shigella sonnei* infection occurred in a household after eating foods in a housewarming party at Pakapol Village, South 24 Parganas District of West Bengal, an Indian state, in November 2016. Here, we report the epidemiological and microbiological findings of this outbreak. Thirty-four people attended the party on November 23, 2016, and had lunch together. The median incubation period from the time of food consumption to the development of acute gastroenteritis was 18.5 h (interquartile range, 16.5–22 h). The overall attack rate was 73% (25/34), and 76% (19/25) of them required hospitalization. All age groups were affected with 100% recovery rate. One served food item was significantly associated with the illness, i.e., tomato salad (risk ratio, 4.14; 95% confidence interval, 1.21–14.13). Among the 12 stool specimens tested, 8 (67%; 8/12) were positive for *S. sonnei*. All *S. sonnei* strains were completely resistant to nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin, and erythromycin, and partially resistant to tetracycline, doxycycline, streptomycin, and trimethoprim/sulfamethoxazole. Pulsed-field gel electrophoresis analysis showed that the recent outbreak strains of *S. sonnei* were clonally related with the locally circulating strains in Kolkata.

INTRODUCTION

Foodborne disease is a major public health problem both in developed and developing countries (1,2). Its outbreak is defined as an incident where 2 or more persons experience similar illness resulting from ingestion of a common food (3). Bacteria are the causative agents of foodborne illness in 60% of cases requiring hospitalization (4). Infection with *Shigella* spp. is one of the major causes of foodborne diseases due to their low infectious doses and person-to-person transmission by the fecal-oral route (5). The risk of transmission and infection increases with poor hand hygiene, ingestion of contaminated food or water, inadequate sanitation and toileting, and overcrowding. Annually, 3–5 billion cases of infectious diarrhea and 1.8 million deaths mainly in young children due to contaminated food and water are reported worldwide (6).

In India, the Integrated Disease Surveillance Program reported 306 foodborne outbreaks in 2014 (7). Foodborne outbreak alone constitutes 20% of all outbreaks reported (7). Unfortunately, reports on the investigation of foodborne outbreaks are extremely limited. The available information on foodborne disease outbreak investigation from 1980 to 2009 indicated that a total of 37 outbreaks affected 3,485 persons (8). In 1984–1985, devastating dysentery outbreaks were reported due to infection with *Shigella dysenteriae* type 1 (9). Although *Shigella dysenteriae* and *Shigella flexneri* are the predominant species in tropical countries, foodborne outbreaks due to *Shigella sonnei* are also being reported in countries like India (9). In 2009–2010, 2 foodborne outbreaks caused by *S. sonnei* were reported in India (9).

In the state of West Bengal, foodborne outbreaks commonly occur. In response to many cases of acute gastroenteritis (AGE) reported to Infectious Disease & Beliaghata General Hospital (ID & BG), Kolkata, in one household at Pakapol Village, Bhangore II block, South 24 Parganas, on November 24, 2016, an investigation was conducted with the following objectives: (i) to confirm the existence of an outbreak; (ii) to describe it in terms of time, place, and person; (iii) to determine the cause of outbreak; and (iv) to recommend control measures.

MATERIALS AND METHODS

Confirmation of foodborne outbreak: All the admitted patients (n = 19) at the ID & BG Hospital were from a single household at Pakapol Village, Bhangore II block, South 24 Parganas, whom we interviewed on November 25, 2016, to confirm the occurrence of the foodborne outbreak. In this outbreak, we defined a case of AGE as any patient who developed diarrhea/dysentery with any of the following symptoms: abdominal pain, fever, and vomiting within 3 days of eating at the housewarming party held on November 23, 2016 (10). The National Institute of Cholera and Enteric Diseases runs a routine hospital-based surveillance at the ID & BG hospital, which was approved by Institutional

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*Corresponding author: mailing address: Division of Bacteriology, National Institute of Cholera and Enteric Diseases, P-33, CIT Rd, Scheme XM, Beliaghata, Kolkata 700010, India. Email: shanta1232001@yahoo.co.in

Ethical committee. Through this surveillance, we could identify this type of sporadic outbreak, and hence, we did not require separate ethical permission to investigate them. Moreover, our findings help the local health authority to take appropriate control and preventive measures. However, data were collected from all party attendees after obtaining consent.

Descriptive epidemiology: We conducted a retrospective cohort study including all individuals who attended the housewarming party. Epidemiologists along with the health workers visited the affected household and surveyed the kitchen area where cooking was done for the party. We collected information on sociodemographic details such as food exposure, time of food consumption, type of illness, date and time of onset of illness, clinical profile, and outcome.

Sample processing at the laboratory: Twelve stool specimens were collected using sterile catheters from the admitted cases and were examined within 2 h in the bacteriology laboratory of NICED for common enteric pathogens (11). Briefly, stools were cultured on MacConkey, Xylose-lysine deoxycholate (XLD), and Hektoen enteric (HE) agars to isolate *Escherichia coli*, *Shigella* spp., and *Salmonella* spp., respectively. Ryan medium and thiosulfate citrate bile salt sucrose (TCBS) agar were used to isolate *Aeromonas* spp. and *Vibrio* spp. respectively. In addition to the direct plating, fecal samples were inoculated into selenite-F and alkaline peptone water to enrich *Salmonella* spp. and *Vibrio* spp., and then sub-cultured on XLD/HE agar and TCBS agar plates, respectively. All the agar plates and enriched broths were incubated at 37°C overnight. In addition to enteric bacteria, enteric viruses (rota- and adenoviruses) were also screened using commercial kits (Tech Lab, Radford, VA, USA) (11).

Sucrose-xylose-lactose non-fermenting red colonies on XLD agar and green colonies on HE agar, suspected as *Shigella* spp., were subsequently tested for fermentation reactions in triple-sugar-iron, lysine-iron, motility-indole-ornithine, simmons citrate, and urea agars. The isolates were identified as *Shigella* spp. using standard methods (12). Biochemically identified *Shigella* isolates were confirmed serologically by slide agglutination using commercially purchased antisera (Denka Seiken Co. Ltd,Tokyo,Japan).

Antimicrobial susceptibility test was performed using commercial antibiotic discs following the manufacturer's instructions (BD). The inhibition zone diameters recorded were resistant to an antimicrobial agent based on the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014). *Escherichia coli* strain ATCC 25922 was used for quality control.

Polymerase chain reaction (PCR) assays: Three typical lactose-fermenting colonies confirmed as *Escherichia coli* by biochemical testing were tested in the multiplex PCR assay to detect the 3 pathogroups of diarrheagenic *E. coli* (i.e., ETEC, EPEC, and EAEC) (2). Virulence genes such as *ipaH* were also detected by a simplex PCR assay using published primer pairs (13). Template DNA was prepared with the growth of the test strains in Luria broth (Difco) supplemented with 0.85% NaCl, and PCR assay was performed using our previously published protocol.

Pulsed-field gel electrophoresis (PFGE): PFGE of *XbaI*-digested genomic DNA of the outbreak *S. sonnei* strains was performed using a CHEF-Mapper (Bio-Rad Laboratories, Hercules, CA,USA) according to the PulseNet standardized protocol for subtyping of *Shigella* species (14). PFGE images were captured using a Gel Doc XR system (Bio-Rad). The gel images were normalized by aligning the peaks of the "Salmonella Braenderup" size standard and analyzed using the BioNumerics software version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium). The degree of banding similarity was determined by comparing the dice coefficient, and the clustering correlation coefficients were calculated using the unweighted pair group method with arithmetic mean.

Data collection and analysis: Epidemiologists along with trained health workers collected all sociodemographic information using institutionally devised acute gastroenteritis case search format. An epidemic curve was plotted by date and time of onset of AGE along with the calculation of age- and gender-specific attack rates. Hospitalization rate was also calculated. The clinical profiles of all AGE cases were recorded. The risk ratio (RR) with 95% confidence interval (95% CI) were calculated to determine the association between food consumption and AGE development. We have used EpiInfo version 7 software (CDC, Atlanta, GA, USA) for data analysis.

The work has been carried out at ICMR - National Institute of Cholera and Enteric Diseases, Kolkata.

RESULTS

Confirmation of foodborne outbreak: All 19 hospitalized AGE cases in the ID & BG Hospital, Kolkata, from a single household of Pakapol Village, South 24 Parganas, provided a history of eating the same food at a house party, resulting in development of AGE.

Descriptive epidemiology: Twenty-five people developed AGE after eating foods at a house party. The last case was reported on November 25, 2016, at 5 am. The median incubation period from the time of food consumption to development of AGE was 18.5 h (interquartile range [IQR], 16.5–22 h) and indicated toward a point source outbreak (Fig. 1).

A total of 34 individuals attended the party on November 23, 2016, and all had lunch together. Foods were cooked in the afternoon. Only the salad was made before serving the foods, which was mainly composed

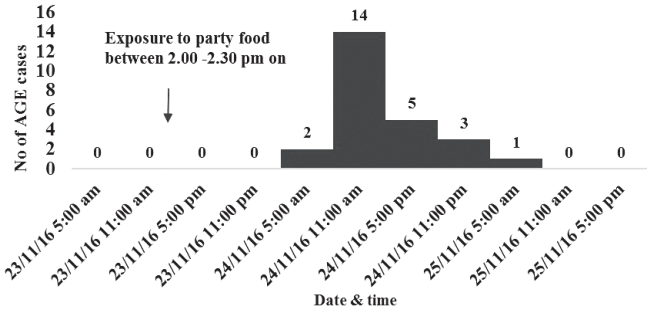

Fig. 1. Distribution of Acute Gastroenteritis Cases, food borne outbreak, Pakapol Village, Bhangore II, South 24 Parganas, West Bengal, India, November 23–25, 2016 AGE, Acute Gastroenteritis.

Table 1. Gender & age specific attack rate of Acute Gastroenteritis in a food borne outbreak, Pakapol Village, Bhangore II, South 24 Parganas, West Bengal, India, November 23–25, 2016

Characteristics	No of confirm cases (*n*)	Population	Attack rate (%)
Age (in years) 3-10	7	9	78
11-25	6	10	60
26-38	6	8	75
39-60	6	7	86
Gender			
Male	12	18	67
Female	13	16	81
Overall	25	34	73

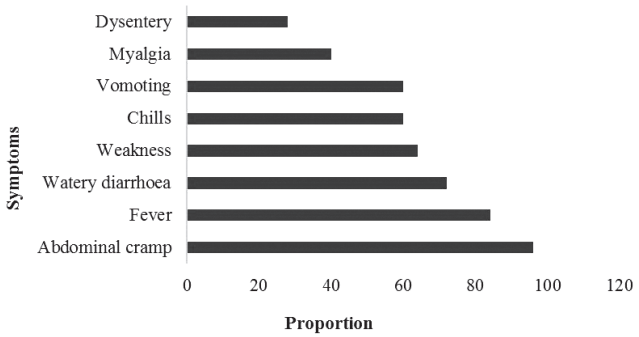

Fig. 2. Clinical profile of 25 Acute Gastroenteritis patients of food borne outbreak, Pakapol Village, Bhangore II, South 24 Parganas, West Bengal, India, November 23–25, 2016.

Table 2. Food items served at the party, Pakapol village, Bhangore II, South 24 Parganas, West Bengal, India, November 23, 2016

Food item		Outcome					
		Developed AGE (25)		Did not develop AGE (9)		RR	95% CI
		n	%	*n*	%		
Tomato Salad	Ate	23	92	2	8	**4.14**	**1.21 – 14.13**
	Did not eat	2	22.2	7	77.8		
Chicken Biriyani	Ate	1	33.3	2	66.7	0.43	0.08 – 2.15
	Did not eat	24	77.4	7	22.6		
Beef Biriyani	Ate	25		7		Infinity	Infinity
	Did not eat	0		2			
Soft drink	Drank	11	78.6	3	21.4	1.12	0.75 – 1.66
	Did not drink	14	70	6	30		
Papad	Ate	12	80	3	20	1.17	0.78 – 1.74
	Did not eat	13	68.4	6	31.6		

CI, Confidence Interval; RR, Risk Ratio; AGE, Acute Gastroenteritis.

of unrefrigerated raw sliced tomatoes from the previous day. Foods were served between 2.00 and 2.30 pm. Only 5 of them consumed the foods between 5 and 7 pm. There was no leftover food in the party. Among them, 25 developed AGE (overall attack rate [AR], 73%), and 76% (19) of them required hospitalization. Table 1. shows the age and sex distribution. Female individuals had higher attack rate (AR among females = 81%). Median age of all the case patients was 25 years (IQR, 10 years – 38 years). No case fatality was reported.

Analytical epidemiology: Out of the 5 food items served in the party, only one food item was significantly associated with the illness. i.e., tomato salad (RR, 4.14; 95% CI, 1.21–14.13) (Table 2).

Clinical profile of AGE case patients: Among AGE cases, 96% (24/25) had abdominal cramp and 84% (21/25) had fever followed by watery diarrhea, dysentery, weakness, chills, vomiting, and myalgia (Fig. 2).

Confirmation of diagnosis/laboratory test: Out of 12 stool samples tested, 8 (67%) were tested positive for *S. sonnei* as a sole pathogen, and no other enteric pathogen was detected in the remaining samples.

All *S. sonnei* strains were resistant to nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin, and erythromycin (100% each), followed by tetracycline (87.5%), doxycycline (87.5%), streptomycin (75%), and trimethoprim/sulfamethoxazole (62.5%). However, all strains were susceptible to ampicillin, chloramphenicol, meropenem, azithromycin, ceftriaxone, cefotaxime, and ceftazidime (100% each) (Table 3). PCR results that identify the virulence genes showed that all *S. sonnei* outbreak strains were positive for virulence-encoding regions of the invasion plasmid antigen H (*ipaH*) gene.

To confirm the clonal relationship, all (*n* = 8) *S. sonnei* outbreak strains and 5 sporadic strains isolated

Table 3. Antimicrobial susceptibility results of *Shigella sonnei* strains found in food borne acute gastroenteritis outbreak, Pakapol Village, Bhangore II, South 24 Parganas, West Bengal, India, November 23–25, 2016

Antimicrobial	Resistance (R) (%)	Intermediate (I) (%)	Susceptible (S) (%)
Ampicillin	-	-	100
Chloramphenicol	-	-	100
Trimethoprime-Sulfamethoxazole	62.5	12.5	25
Ciprofloxacin	100	-	-
Norfloxacin	100	-	-
Gentamycin	-	-	100
Nalidixic acid	100	-	-
Neomycin	-	12.5	87.5
Streptomycin	75	25	
Tetracycline	87.5	-	12.5
Erythromycin	100	-	-
Ofloxacin	100	-	-
Meropenem	-	-	100
Ceftriaxone	-	-	100
Azithromycin	-	-	100
Doxycycline	87.5	-	12.5
Cefotaxim	-	-	100
Ceftazidime	-	-	100

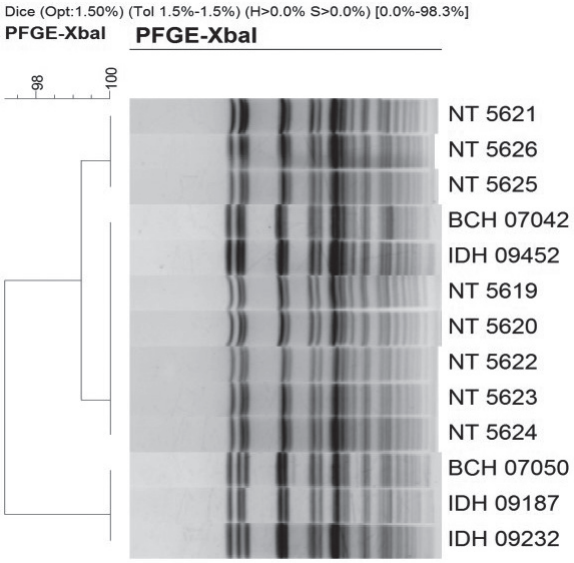

Fig. 3. Pulsed-field gel electrophoresis (PFGE) analysis of *XbaI*-digested genomic DNA of *Shigella sonnei* strains isolated during the outbreak along with the strains isolated in the surveillance study in Kolkata, India during 2016.

from hospitalized diarrheal patients during 2016 in Kolkata were included in the PFGE analysis. The dendrogram analysis showed that the recent outbreak *S. sonnei* (NTs) strains were clonally related with sporadic *S. sonnei* strains (BCHs and IDHs) (less than 2 bands' difference) (Fig. 3).

DISCUSSION

Based on epidemiological and laboratory evidences, it was concluded that the event was a foodborne acute gastroenteritis outbreak caused by fluoroquinolone-resistant *S. sonnei* infection, which started on November 24, 2016, at around 5 am, and the last case was reported on November 25, 2016. It affected many of the party attendees who consumed food. More than two-third of the cases required hospitalization, and no fatality was reported. Due to low dose of infection and conducive environmental factors, *S. sonnei* could spread through food, water, and direct person-to-person transmission which triggered this outbreak; however, the concern that it might cause extensive secondary transmission from the same source remains (15).

Although food samples were not available for laboratory testing in this outbreak, the history of using unrefrigerated leftover cut tomatoes from the previous day to prepare the salad for the party might explain the association between its consumption and development of illness. Moreover, chicken, beef biriyani, and fried chips were prepared on that afternoon. In 2008, similar type of *S. sonnei* outbreak was reported in Austria where consumption of salad also led to illness (16).

Food handlers commonly play an important role in transmission of foodborne infections as they may introduce pathogens into foods during preparation, processing, or distribution (10). However, in this outbreak, none of the food handlers gave any recent (within the last 2 weeks before the party) history of diarrhea/dysentery during the interview. Another important concern reported in this investigation was inadequate sanitation and hygiene of the household. The household had a pucca toilet with a closed septic tank, which was situated only 5–7 meters away from the hand water pump, the source of water used to clean utensils. Moreover, the household had no proper drainage system, and washing hands with soap before eating was not regularly practiced among the party attendees. In this type of conditions, where hygiene and sanitation are inadequate, common house flies, particularly *Musca domestica*, may serve as a vector for bacterial transmission (17). All these factors possibly played important roles in the occurrence of this outbreak, caused by multiple antibiotic-resistant *Shigella* which was endemic in that area.

Most of the *S. sonnei* isolates were resistant to commonly used antibiotics, such as ciprofloxacin, ofloxacin, and norfloxacin, which was concerning from the treatment and control perspectives. Several *Shigella* outbreaks that were highly resistant to ciprofloxacin, norfloxacin, and ofloxacin were reported from different parts of India (18). In 1990, ciprofloxacin was recommended as the drug of choice to treat shigellosis (19). However, overuse and misuse of this drug led to the emergence of resistance against it (17). Recently, another ciprofloxacin-resistant *S. sonnei* outbreak was reported in South Korea (20). Even outbreaks of *S. sonnei* with resistance to 3rd-generation cephalosporins and azithromycin were also reported in other Asian countries (15). Emergence of multi-drug resistant *S. sonnei* isolates in South and Southeast Asian countries imposes potential threat of causing many outbreaks in the future, which may be devastating if antibiotics are not used prudently (21–23).

This outbreak investigation had few limitations. First, food samples could not be tested for any pathogens as there was no leftover food during investigation. Second, we could not examine water samples from the household due to logistic constraints. Finally, the results of this investigation underscored the need for adequate precautions to prevent the contamination through the raw cut salad, especially tomatoes, and to improve personal hygiene and living conditions.

We conclude that the probable source of infection was contaminated tomato salad consumed during the house party on November 23, 2016. *S. sonnei* was the bacteria responsible for this outbreak. Substantial proportion of party attendees were affected, and all of them recovered after proper management.

The following hygiene and infection control practices were recommended to prevent such episodes in future: (i) practicing hand-washing with soap and water after defecation and before preparing, serving, or eating food, (ii) creating a proper drainage system to avoid stagnation of waste water, (iii) storing food items at proper temperature and also serving hot foods, and (iv) not using antibiotics without consulting a physician for any diarrhea/dysentery episode.

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Conflict of Interest None to declare.

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